

Competitive exclusion of *Salmonella* serovar Typhimurium from the gut of early weaned pigs

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Introduction

Strategies to curb infection of pigs by *Salmonella* are sought throughout the farm to table continuum. Competitive exclusion (CE), which involves colonizing the gastrointestinal tract of young animals with a healthy gut microflora, has been shown to be an attractive strategy for protecting poultry from salmonellae infections (4-7, 14, 16-17, 19-20, 23). More recent evidence suggests that CE may enhance resistance of young pigs against infections by *Salmonella* serovar Choleraesuis (2, 9, 15, 21), the serotype responsible for the vast majority of swine salmonellosis cases in the United States (22). *Salmonella* serovar Typhimurium can also infect and cause disease in swine and may be considered a greater food safety concern than *Salmonella* Choleraesuis but until now, reports regarding the effect of CE treatment on colonization resistance of early weaned pigs to this serotype have been few if not nonexistent.

Materials and Methods

The litters from two sows were farrowed at the USDA Food Animal Protection Research Lab in 1.5 m x 2.1 m Hog Slat Inc., (Newton Grove, NC, USA) farrowing crates. The piglets of one litter (n = 9) were treated orally with 5 ml of a porcine derived CE culture at birth and at weaning. The CE culture, designated as porcine continuous flow culture 1 (pCF1), was propagated from gut contents of a healthy 6 week-old pig and was maintained in steady state via continuous flow culture methodology (16). The fermentor was operated at 39°C at a dilution rate of one volume turnover/day (1,150 ml/day) via infusion of sterile, anaerobic Viande Levure broth (3). Those piglets of the other litter (n = 6) were treated likewise with 5 ml of sterile Viande Levure broth. Both litters were weaned at 14 days of age to separate concrete floored pens of approximately six square meters in dimension and were challenged at 15 days of age with 2.2 to 3.6 x 10⁷ colony forming units (CFU) of a nalidixic acid and novobiocin resistant *Salmonella*

Typhimurium. The challenge dose was determined via enumeration on Brilliant Green agar (BGA) containing 25 µg NOV/ml, (BGA/NOV). Pigs were phase fed rations which met or exceeded NRC requirements (18).

Duplicate fecal specimens (1 to 2 g) collected from the maternal dams 48 hours prior to and after farrowing were each cultured for wildtype salmonellae via initial culture in 20 ml of either GN-Hajna or tetrathionate broth, then further enrichment (100 µl of each) in 5 ml Rappaport-Vassiladias (RV) broth. Selective differentiation was accomplished on Brilliant Green agar BGA/NOV. Beginning at weaning, rectal swabs were collected daily from each piglet for qualitative recovery of salmonellae. Swabs collected the two days immediately prior to challenge were cultured for wildtype salmonellae as above except each swab was cultured in 5 ml GN-Hajna or tetrathionate broth. Swabs collected post challenge were cultured for the NOV/NAL resistant challenge strain of *Salmonella* Typhimurium in tetrathionate and RV broth as above and selective differentiation was accomplished using BGA supplemented with both 25 µg NOV/ml and 20 µg NAL/ml (BGA/NOV-NAL). Piglets were euthanized eight days post challenge via injection with sodium pentobarbital and ileocolic lymph nodes and cecal contents were collected by necropsy. These specimens (1 to 2 g) were cultured qualitatively for the challenge strain as above except in 20 rather than 5 ml tetrathionate broth. Cecal contents were also cultured quantitatively by plating serial 10-fold dilutions directly to BGA/NOV-NAL. All incubation steps were carried out at 37°C for 18 to 24 hr. Plates were examined for colonies exhibiting typical salmonellae morphology and suspect colonies were confirmed via serum agglutination using *Salmonella* Antiserum Poly A I-IV. Since no salmonellae were recovered from any of the prechallenge specimens, we conclude that the piglets were colonized by the challenge strain only.

Results

All piglets were observed to shed the challenge strain of *Salmonella* Typhimurium at least once post challenge. Shedding was most frequent the first three days post challenge and then declined markedly thereafter, but more so for those piglets treated with the CE culture (Figure 1). At necropsy 8 days post challenge, our *Salmonella* Typhimurium strain was recovered from ileocolic lymph nodes of 56% (5 of 9) of the piglets treated with the CE culture and from 50% (3 of 6) of the placebo-treated piglets. Our challenge strain was recovered from cecal contents of 5 of the 9 piglets treated with the CE culture and from all 6 placebo-treated pigs. Average concentration of *Salmonella* Typhimurium in cecal contents was 0.8 CFU/g for those treated with the CE culture and 1.5 CFU/g for those treated with the placebo.

Discussion

Salmonellae-contaminated feces is likely a major source of infection to previously non-infected animals (13) and evidence suggests that both the concentration and incidence of salmonellae shedding influence disease transmission and the establishment of carrier animals (1, 10-12). Repeated exposure to contaminated feces may prolong fecal shedding (8) which may thus prolong carriage of the pathogen within an infected herd. The cumulative incidence of *Salmonella* Typhimurium shedding in the present study was less for the CE-treated piglets (44%) than for those treated with the placebo (77%) thus suggesting a protective effect of CE. Moreover, cecal colonization by *Salmonella* Typhimurium was also reduced among the CE-treated pigs (56% of the CE-treated versus 100% of the placebo-treated piglets). Whether or not CE treatment in this study would correspond to reduced transmission of *Salmonella* Typhimurium to other noninfected pigs is not discernable. However, evidence also presented at this symposium, (see Anderson, R. C. et al., Effect of Competitive exclusion on transmission of *Salmonella* serovar Choleraesuis between early weaned pigs) suggests that CE treatment did reduce horizontal transmission of *Salmonellae* Choleraesuis between infected and previously uninfected pigs.

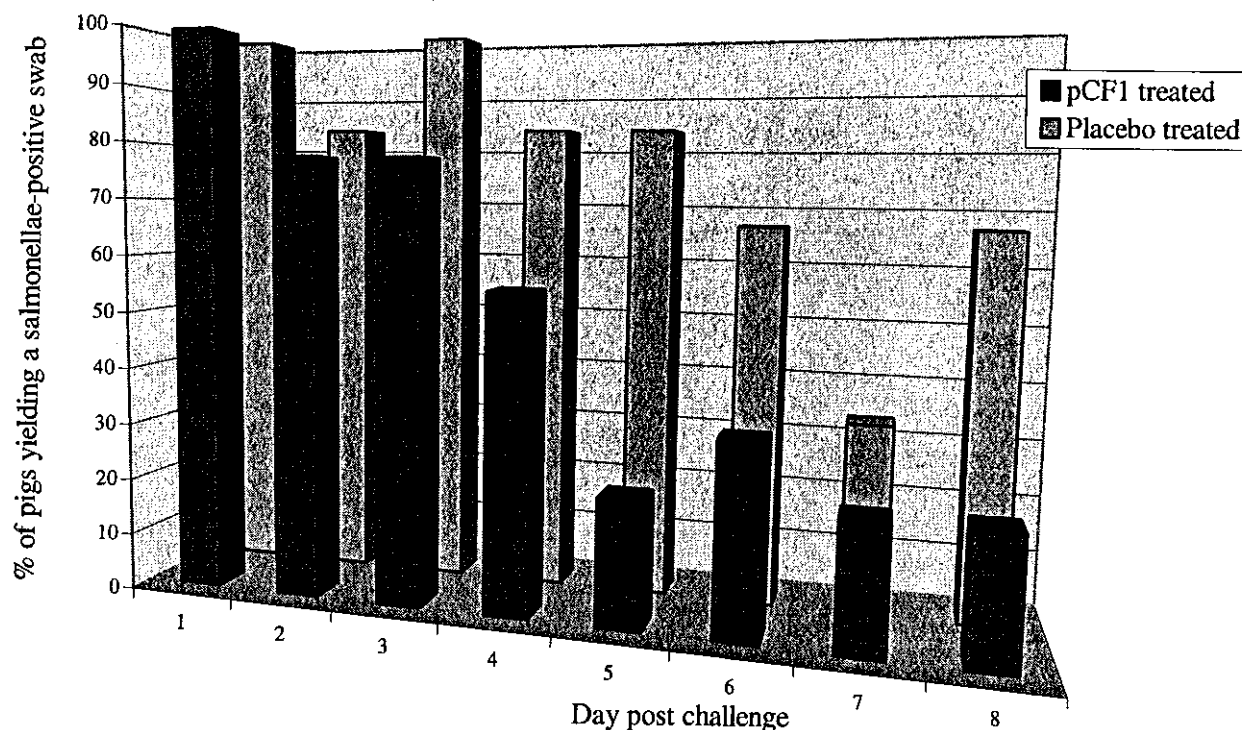


Figure 1. Daily incidence of shedding of *Salmonella* Typhimurium as determined by qualitative culture of rectal swabs.

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